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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP # 3F2896 and FAP # 3H5398. Pirimiphos-methyl on stored peanuts. Evaluation of analytical method and residue data.

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THRU: Charles L. Trichilo, Chief
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TO: Jay Ellenberger, Product Manager No. 12
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ICI Americas Inc. proposes that tolerances for residues of the insecticide pirimiphos-methyl, O-(2-diethylamino-6-methylpyrimidin-4-yl) O,O-dimethylphosphorothioate, the metabolite O-(2-ethylamino-6-methylpyrimidin-4-yl) O,O-dimethylphosphorothioate and, in free and conjugated form, the metabolites 2-diethylamino-6-methylpyrimidin-4-ol 2-ethylamino-6-methylpyrimidin-4-ol and 2-amino-6-methyl-pyrimidin-4-ol be established in or on the following raw agricultural commodities:

Peanuts	25	ppm
Peanut hulls	125	ppm
Milk	0.5	ppm
Meat, fat and meat byproducts or cattle, goats, hogs, horses, and sheep (except liver and kidney)	0.15	ppm
Liver/liver byproducts	1.0	ppm
Kidney/kidney byproducts	2.0	ppm
Poultry	4.0	ppm
Eggs	0.5	ppm

The petitioner also proposes that a food additive tolerance of 50 ppm be established on peanut oil.

At present, a 5 ppm permanent tolerance involving only the parent compound has been established on kiwifruit; no other permanent tolerances have heen established. If the tolerances proposed in this petition and/or in a co-pending petition (PP#3F2897/3H5399) are established, then the present tolerance established on kiwifruit will be revised to include the major metabolites of pirimiphos-methyl.

Conclusions

1a. The nature of the residue in peanuts is not fully understood for the establishment of permanent tolerances. The petitioner should attempt base and enzymatic hydrolyses, etc. in an effort to release the bound or polar residues and identify the aglycone. The bound and polar residues referred to are those unidentified residues (especially those in seed coat samples and meats) displayed on the various TLC chromatograms.

1b. The nature of the residue in animals is not fully understood. Further work is needed for the characterization of unknown residues in meat, milk, poultry and eggs (see also the Nature of the Residue Section of this review).

2a. We cannot conclude, at this time, that adequate methodology is available for regulatory purposes. Our conclusion depends on the outcome of the petitioner's further characterization of unidentified residues. Once the methodology is ready for regulatory purposes, we will make a request for a method trial. However, the petitioner should be informed that if the present methodology involving a surface extraction (see Method No. 47 for pyrimidine metabolites) of grain samples were accepted for enforcing the proposed tolerances on stored grain, it would not be acceptable for the extraction of pyrimidine metabolite residues from grain samples resulting from the field use of pirimiphos-methyl.

2b. The petitioner will need to delete the need for an internal standard from his proposed methodology. Internal standards are considered undesirable for regulatory purposes.

3a. We confirm our conclusion as stated in our review of PP# 9G2154/FAP 9H5201 and its subsequent amendments that the following proposed pirimiphos-methyl tolerance levels are adequate to cover initial residues as applied to the peanuts: (1) peanuts - 25 ppm, (2) peanut hulls - 125 ppm, and (3) peanut oil - 50 ppm; the reason being that these are calculated residue (tolerance) levels.

3b. Even though the tolerances are numerically adequate, the tolerance expression may need to be revised to include additional metabolites.

4a. At this time, we can not draw conclusions on the adequacy of the proposed meat, milk, fat, and meat byproducts tolerances; we need to understand better the nature of the residue in plants and animals. If metabolism is different in plants versus animals, a feeding and metabolism study with the plant residues of concern may be needed.

Also, the petitioner should note that there was a 2X concentration of residues going from milk to butter. Therefore, there may need to be a tolerance proposal on a milk fat basis.

4b. We can not draw any conclusions on the adequacy of the proposed tolerances on poultry, meat, and eggs until questions relating to the nature of the residue in plants and animals/poultry have been resolved.

4c. Residue data on poultry fat are needed.

4d. Tolerances will need to be proposed on poultry meat byproducts and poultry fat.

5. An International Residue Limit Status sheet is attached to this review. No Canadian and Mexican limits/tolerances have been established on peanut and animal commodities. We observed in the WHO Pesticide Residue Series No. 4 (1974 Evaluation of Some Pesticide Residues in Foods) that the (Codex) use on peanuts is similar to the proposed U.S. use on peanuts. However, the Codex tolerances are much lower than the U.S. proposed tolerances, presumably, because the Codex tolerances do not include the hydroxypyrimidine metabolites. The tolerances are thus not numerically compatible.

Recommendations

At this time, we recommend against establishment of the proposed tolerances for reasons given in conclusions 1a, 1b, 2a, 2b, 3b, 4a, 4b, 4c, and 4d.

Detailed Considerations

Manufacture and Formulation

The manufacturing process for pirimiphos-methyl is discussed in our 3/29/79 review of PP#9G2154/FAP#9H5201.

Technical pirimiphos-methyl is a minimum of 90% pure. Impurities in the technical material include [redacted]

[redacted]

The formulation to be used on peanuts is ICI America's Actellic 7E Insecticide which contains 7 lbs of technical pirimiphos-methyl/gallon. All inerts in the formulation are cleared under Section 180.1001.

Proposed Use

To control insects in stored peanuts apply 0.612 lb active ingredient in 5 gallons of water per 15 tons (30,000 lbs) of peanuts. This would result in ca. 20 ppm initial deposit on the peanuts. Application should be made as peanuts enter storage utilizing a coarse, uniform, low pressure spray.

Nature of the Residue

Plants

Metabolism studies on stored wheat, rice, and peanuts were discussed in our (R.B. Perfetti and R.J. Hummel) 3/29/79 review of PP#9G2154 and FAP#9H5201 and our (N. Dodd). 4/10/80 review of PP#9G2200 and FAP#9H5217. For permanent tolerances, the previously mentioned peanut metabolism studies are reviewed below.

In an in-shell peanut metabolism study, ^{14}C-labeled pirimiphos-methyl was applied at a rate of approximately 31 ppm as a spray. The residue data show that at 91 days after treatment, 76.8% (50.2% by methanol, 23.8% by hexane/acetone, and 2.8% by methanol-hexane/acetone extracts) of the total residues in peanut meats could be extracted, and by GLC, 38.1% of total activity was identified as pirimiphos-methyl. No other residues were quantified by GLC analysis; thus, 61.9% of the total residues were not accounted for. When the methanol extracts (which extracted only 50.2% of the total residues) were subjected to TLC analysis, metabolite IV (2-diethylamino-6-methylpyrimidin-4-ol was the major metabolite existing at a ratio of about 1:5 (14.3%:75:3%) with the parent compound. The percentages of the other metabolites (Metabolites II, III, V, and VI) within the methanol extracts ranged from 0.4 to 2.3% of the extractable residues. However, no TLC analyses were presented on the hexane/acetone and methanol/hexane/acetone successive extracts.

Unextractable radioactivity in peanut hulls increased from 1% at 0 day to a maximum of 16% of the total radioactivity after 91 days. It is somewhat vague as to whether the

86% (100%-16%) extractable residues from hulls resulted from the same succession of extractions (methanol followed by hexane/acetone and methanol-hexane/acetone extractions) as was done to obtain the extractable residues (76.8%) from peanut meats in which case the methanol extractable residues represented 50.2% of the total residues. The report shows that methanol extractable residues of peanut hulls were subjected to TLC. Of the methanol extractable residues at 91 days, TLC analyses showed the total residue to be about 75% pirimiphos-methyl, 14% metabolite IV and 2.3% or less for each metabolite II, III, V, or VI.

Thus, at 91 days, the unidentified residues are the initial 16% unextracted plus about 4% lost/unidentified during the TLC process plus the possible amount extractable by the successive hexane/acetone solvent mixture plus also the possible amount that could be extracted by a methanol-hexane/acetone mixture. Thus, the unaccounted for radioactivity on peanut hulls could very well parallel the unaccountable radioactivity observed for peanut meats if the same succession of extractions was carried out for both commodities.

Additional Consideration. In the introduction section of the Report 0127B, Analysis of Seed Coats from Pirimiphos-methyl Treated In-Shell Peanuts, submitted in the present submission, the petitioner indicates that the extractable radioactivity presumedly 2 mg/kg-1 in the peanut kernels was fully characterized as pirimiphos-methyl 6 months after treatment; reference is made to Report RJ 0086A submitted in amendment 9/6/79 to PP# 9G2154. In the RJ 0086A report, the peanut kernels were extracted with a solvent mixture of hexane/acetone. However, the RJ 0086A report indicates that analyses by GLC showed the pirimiphos-methyl residue in the hexane/acetone extracts of Bin 1 and Bin 2 kernel samples to be 0.506 and 0.306 mg/kg, respectively. The preceding values represent about 25% of 2 ppm.

Analysis of Seed Coats from Pirimiphos-methyl Treated In-Shell Peanuts.

Metabolism data submitted in the present petition and also submitted in a co-pending petition #3F2897 deal with the characterization of radioactive residues on the seed coats of peanuts which have been treated with ^{14}C-pirimiphos-methyl and stored for 6 months. Metabolism data for the residues on the kernels and shells of these peanuts are discussed above.

The residue data indicate that 86-90% of the total radioactivity in seed coats was extracted into methanol; we note that this percentage of extraction is higher than the 50.2 percentage observed on peanut meats. Later, when the prepared concentrated methanol extract (now representing

68.2% of the total residue; presumedly, 20-22% of the radioactivity as pirimiphos-methyl has been removed by hexane) was subjected to TLC analyses, about 58% of the total residue on the TLC plate was Metabolite II, 0.6% Metabolite IV, 38.3% remained at the origin, and 3.5% of the radioactivity was located on the remainder of the chromatogram. Thus, of the prepared concentrated methanol extract, 41.8% of the residue on the TLC plate was not identified. When we add the preceding percentage to the originally unextractable residues, the terminal metabolism residue data is about 50% characterized.

Comments on the Plant Metabolism Studies

Since no permanent tolerances involving metabolites have been established, we must ensure that all questions on the metabolism processes are resolved.

The residue data on the peanut meats show that at 91 days 76.8% of the total residue in peanut meat could be extracted by the following successive extractions: methanol-50.2%, hexane/aceton-23.8%, and methanol-hexane/acetone-2.8%. The methanol extractable residues were examined in some detail by TLC and GLC. We estimate that less than 50% of the radioactivity in peanut kernels has been adequately characterized.

As for peanut hulls and peanut seed coats, the metabolism data also indicate that about 50% of the terminal residues were adequately characterized provided that the same succession of extraction solvents were used.

The petitioner should attempt base and enzymatic hydrolyses, etc., in an effort to release the bound or polar residues and identify the aglycone. We would expect base to react unfavorably on any of the unidentified residues containing phosphorus; however, it may have little effect on uncharacterized residues containing only the pyrimidine moiety. Specific enzymes may well release, for example, the compound O-2-amino-6-methylpyrimidin-4-yl O,O-dimethylphosphorothioate (which has been given little consideration) without doing any damage to the moiety containing phosphorus.

It is our understanding that TOX wants the assurance that CHE inhibiting metabolites would be less than 10 ppm. For the establishment of permanent tolerances, more of the unidentified residues would need to be characterized in order to ensure that the 10 ppm level for the CHE inhibiting metabolite level is not exceeded.

Animals

Several metabolism studies in goats, cows, chickens,

rats, and dogs were discussed in our 3/29/79 review of PP# 9G2154, and they may be referred to therein.

One of the goat experiments (RJ 0027A- date of issue: 8/4/78), in brief, involved twice daily oral doses of ^{14}C-pirimiphos-methyl totaling 30 ppm in the diet for seven days. Milk was sampled twice daily. Urine and feces were collected daily. Four hours after administration of the last dose, the animals were slaughtered and tissues were sampled.

Radioactive residues in milk appeared to plateau at day 3, showing a maximum level of 0.181 ppm. The percentage of the total dose found in milk was 0.342. Significant amounts of radioactivity were found in the urine and feces (ca 49% of total dose). Radioactive residues ranged from 0.05-0.06 ppm in spleen, 0.75 to 0.83 ppm in kidney, 0.33 to 0.38 ppm in liver, 0.08 ppm in the heart, 0.03 to 0.05 ppm in muscle, 0.05 to 0.07 ppm in the brain, and 0.09 to 0.11 ppm in the lungs. In all, only 58% of the total dose was recovered. No explanation was given for this.

When milk samples were extracted with acetone and acetonitrile, 66 to 72% of the radioactivity in milk could be extracted. Of this, 38 to 47% could be tentatively identified as a mixture of compounds (I-IV). When milk was extracted with methanol, 89 to 94% of the radioactivity was extracted. Of this, 42 to 59% was identified by reverse isotope dilution as 2.3 to 2.87% hydroxy pyrimidine (IV), 30 to 40% desethyl hydroxy pyrimidine (V), and 11 to 16% hydroxyamino pyrimidine (VI). No parent was identified in these milk samples. Radioactive residues in urine and feces were 95 and 92% extractable with methanol. Most of this radioactivity was identified as metabolites V, VI, and IV in decreasing order of abundance. Methanol could extract the following percentages of total radioactivity present in the tissues: kidney (93%), liver (64%), lungs (74%), and heart (93%). Muscle tissue samples were not extracted. Radioactive residues in kidney, liver and heart were found to consist of 74, 54 and 56% of a mixture of metabolites V, VI and IV in decreasing order of abundance. Radioactive residues identified in the lung were 48% of V, 56% of VI, and 6% of IV. In all, the percentage of the total radioactivity in these tissues identified was 71% in kidney, 35% in liver, 81% in lung and 61% in heart. No parent was identified in these tissues.

A goat metabolism study (RJ 0144B - study conducted 2/18/80 - date of issue 7/9/80) submitted in the present petition involved twice daily oral doses of ^{14}C-pirimiphos-methyl totaling 30 ppm in the diet for seven days. Only the radioactive residues found in the liver and kidney of a goat were discussed in this submission.

A radioactive residue of 0.25 - 0.3 ppm of ^{14}C-pirimiphos-methyl equivalents was detected in the liver, and a radioactive residue of 0.59 - 0.7 ppm equivalents was reported in the kidney. Residues that will be referred to are listed below:

Parent = 0-2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate
I = 0-2-ethylamino-6-methylpyrimidin-4-yl 0,0-dimethyl phosphorothioate
II = 2-diethylamino-6-methylpyrimidin-4-ol
III = 2-ethylamino-6-methylpyrimidin-4-ol
IV = 2-amino-6-methylpyrimidin-4-ol

Residues in Liver: The report indicates that 20g liver samples were first extracted with hexane; this solvent extracted about 7%, of the total residue. The residues in the hexane extract were identified on TLC chromatograms as pirimiphos-methyl (26-29%), compound I (4.7%), compound II (12.5%), and compound IV (0.4%). The remainder of the unidentified residues on the TLC plate ranged from 67-87%. The pellets from the hexane extractions were then extracted with aqueous methanol, and this extract contained about 68% of the total residue.

Overall, the petitioner indicates the following:

> "78.8% of the radioactive residue was extracted using hexane and aqueous methanol. Analysis of these extracts showed that pirimiphos-methyl, Compound II, Compound III and Compound IV represented 5.9%, 2.6%, 18.1% and 16.4% respectively of the total radioactive residue. These results support those obtained by reverse isotope dilution for compounds II, III and IV. The remainder of the radioactivity in these extracts was found to consist of at least five compounds, which were present in approximately equal amounts.
>
> When the methanol extract (C) (representing 68.1% of the radioactive residue) was subjected to acid reflux (2M HCl, 1 hour) there was no significant increase in the amounts of compounds II, III and IV. The liver pellet which remained after extraction with hexane and aqueous methanol, contained 22.4% of the total radioactive residue. When the pellet was refluxed in acid (1M HCl, 1 hour) 9.7% of the total radioactive residue was extracted. This extract was found to contain Compound II, Compound III and Compound IV (representing 1.1%, 3.7% and 1.1% of the total radioactive residue respectively, i.e. 13.7% of the total hydroxypyrimidines detected). The remainder of the extracted radioactivity was found to consist of at least three compounds."

Our Comments on the Residues Found in the Liver.

Initially, the liver contained 0.25 - 0.3 ppm pirimiphos-methyl equivalents; the total radioactive count was 2.19 x 10^5 dpm. When we add all the percentages of the radioactivity representing the parent and compounds I, II, III and IV (5.9% + 2.6% + 18.1% + 16.4% + 1.1% + 3.7% + 1.1%), the total radioactivity accounted for is 48.9%. The petitioner makes reference to the remainder of the radioactivity in these extracts (hexane-methanol) as consisting of at least 5 compounds. If these compounds are complexes, the petitioner may want to treat them with hydrolyzing agents (base and/or enzymes) other than acid, i.e., additional work should be conducted to attempt to identify the remaining radioactivity (ca. 50%).

Residue in Kidney: Twenty grams of kidney samples were first extracted with hexane; this solvent extracted about 2% of the total residue (0.59% - 0.7 ppm); extractable residues were compound II and a large amount of an unknown compound.

The petitioner reports further the following:

> "The methanol extract (G) contained 79.3% of the radioactive residue. Analysis of this extract showed that Compound II, Compound III and Compound IV represented 7.1%, 32.6% and 15.1% respectively of the total radioactive residue. These results support those obtained by reverse isotope dilution for Compounds II, III and IV. The remainder of the radioactivity in this extract was found to consist of at least six compounds which were present in approximately equal amounts.
>
> When the methanol extract (G) was subjected to acid reflux (2M HCl, 1 hour) there was no significant increase in the amounts of Compounds II, III and IV.
>
> The kidney pellet which remained after extraction with hexane and aqueous methanol, contained 13.0% of the total radioactive residue. When it was refluxed in acid (2M HCl, 1hour) 9.5% of the radioactive residue was extracted. This extract was found to contain Compound II, Compound III and Compound IV (representing 0.8%, 2,7% and 1.9% of the total radioactive residue respectively, i.e. 9.0% of the total hydroxypyrimidines detected)."

Our Comments on the Residues Found in the Kidney.

When we add all the percentages of the radioactivity representing the parent and compounds I, II, III, and IV

(7.1%, 32.6%, 15.1%, 0.8%, 2.7%, and 1.9%), the total radioactivity accounted for is 50.2%. The petitioner makes reference to the remainder of the radioactivity in the methanol extract as consisting of at least 6 compounds.

As mentioned above, under Our Comments on the Residues Found in the Liver, the petitioner should exhaust all means such as base and enzymatic hydrolyses, etc., in order to present a better understanding about the 50% unaccounted for radioactive residues. If any of the 6 unknown compounds mentioned above are metabolites other than compounds I, II, III, and IV, we will need to know their identities in our consideration for the establishment of permanent pirimiphos-methyl tolerances.

Studies with chickens reflected the following conditions. Three hens were given a single oral dose of 2, 9, or 20 mg (2-^{14}C) pirimiphos-methyl. Eggs and excreta were collected for 8 days. In a second study, three hens were given twice daily doses of (2-^{14}C) pirimiphos-methyl for 28 days at a dietary level of 4 ppm. Eggs were sampled throughout the period. The birds were sacrificed at the conclusion of the 28 days and muscle tissue samples were taken. In a third study, three hens were fed radiolabeled pirimiphos-methyl at 32 ppm in the diet for 7 days. Again, eggs were sampled throughout the study, and muscle tissue was sampled after the hens were sacrificed.

The eggs of hens fed the single oral dose of pesticide showed highest levels of radioactivity on day 1 and the higher the feeding level the higher the residue. The maximum radioactive residue was 0.67 ppm for the hen fed 20 mg of material. The excreta of one hen 24 hours after dosage showed parent (I), desethyl hydroxy pyrimidine (V), and hydroxyamino pyrimidine (VI) at 4.7, 25, and 31% of the administered dose, respectively.

Radioactivity in the eggs of hens fed 4 ppm pirimiphos-methyl for 28 days plateaued at ca. day 10. The maximum radioactive residue in eggs was 0.039 ppm. Residues in egg whites were higher initially, but as the study progressed the levels in the two fractions became essentially equal. When radioactivity in egg whites and yolks was partitioned with water, 90 and 86% of the activity, respectively, transferred to the aqueous phase indicating the possible presence of water soluble metabolites and conjugates. No radioactivity was found in the egg shells. Maximum radioactive residues in the muscle tissue of these hens at the end of the study is 0.30 ppm.

The hens fed 32 ppm of 2-^{14}C-pirimiphos-methyl for 7 days showed a maximum radioactive residue in tissue of 0.41 ppm. Gas chromatographic analysis of these tissues showed no parent or oxy pirimiphos-methyl (III). Radioactive residues in the eggs of these hens increased throughout the study; maximum residue observed was 0.15 ppm. Residues were again initially higher in the white but levels evened out by the end of the experiment. Gas chromatographic analysis of the eggs showed a maximum of 0.009 ppm of parent in eggs sampled on day 6. No radioactivity was found in any shells.

In response to a review of PP#9G2154, the petitioner has submitted a radiolabeled poultry feeding study. ^{14}C ring-labeled pirimiphos-methyl was fed to laying hens at a rate equivalent to 126 ppm in the diet. Eggs were collected throughout the treatment period. The fate of pirimiphos-methyl was investigated in only eggs and breast muscle. The egg yolks and albumin (whites) were analyzed separately. The yolks and whites were extracted with several different solvent systems and the various metabolites separated on a variety of chromatographic systems. The levels of activity reported in egg yolks and whites ranged as high as 0.08 and 0.06 ppm, respectively. At least 7 different compounds were found in the activity extracted from the whites; of this, 2-ethylamino-6-methylpyrimidin-4-ol and 2-amino-6-methylpyrimidin-4-ol comprised 12-24% and 13-15%, respectively.

A trace of 2-diethylamino-6-methyl-pyrimidin-4-ol and at least four additional polar metabolites, none of which represented more than 20% of the activity, were also found. Acid hydrolysis of the extract yielded 2,4-dihydroxy-6-methyl-pyrimidine which accounted for an additional 8% of the activity. A similar residue pattern was found in yolks. Thus, about 50% of the activity present in the eggs was identified, with most of the remainder characterized as polar metabolites.

The residue levels reported for the breast muscles of the dosed birds ranged as high as 0.59 ppm. Approximately 90% of the activity was extracted with water and subsequently chromatographed on a thin layer plate. About half of the recovered activity remained at the origin, which indicated a highly polar composition. Of the activity, 27 and 9% was shown to be 2-amino-6-methyl-pyrimidin-4-ol and 2-ethylamino-6-methyl-pyrimidin-4-ol, respectively. Traces of 2-diethyl-amino-6-methylpyrimidin-4-ol were also detected. After hydrolysis of the extract, 2-amino-6-methyl-pyrimidin-4-ol comprised 73% of the residue. Its identity was confirmed by both mass spectrometry and isotope dilution techniques. Thus, approximately 74% of the activity in breast muscle can be characterized as either free or conjugated 2-amino-6-methyl-pyrimidin-4-ol and 2-ethylamino-6-methyl-pyrimidin-4-ol.

This poultry metabolism study demonstrates that the free and conjugated hydroxypyrimidine metabolites comprise the majority of the residue in eggs and in poultry muscle tissue.

Our Comments/Conclusions on the Poultry Metabolism Study.

Very little information is presented on the makeup of the terminal residues in poultry and eggs. Gas chromatographic analyses of tissues showed no detectable residues of parent or oxy pirimiphos-methyl (III) in tissue and only small amounts of parent in eggs despite significant amounts of radioactivity being observed in these commodities. About 50% of the activity present in the eggs was identified.

We conclude that the characterization of the residue in poultry and eggs is not adequately understood for the establishment of permanent tolerances. The petitioner should attempt to release bound and polar residues by base and/or enzymatic hydrolyses, etc., in an effort to identify possible aglycones.

Analytical Methods

(I.) Method for pirimiphos-methyl and its phosphorus-containing metabolites.

ICI's Residue Analytical Method No. 11A is used for the determination of the residues of pirimiphos-methyl, O-2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate, and O-2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphate (the latter metabolite was not recommended for inclusion in the tolerance expression for temporary tolerances) in crops, milk, and animal tissues. The declared limit of detection is 0.01 ppm for grains.

Grain samples are extracted by macerating with 20% acetone in n-hexane. The extract is washed with water, and an aliquot may be injected into a gas chromatograph equipped with a flame photometric or thermionic detector. Extracts of grain samples may be cleaned up by following an acetonitrile-n-hexane partition before analyses. Oil samples are dissolved in n-hexane and then partitioned with acetonitrile.

Animal tissues are extracted by warming with n-hexane, and milk is extracted by using a mixture of acetone and acetonitrile. The declared limit of detection is 0.005 ppm for milk.

An additional cleanup using solid-liquid chromatography on Florisil may be used, if necessary, following n-hexane-acetonitrile partition.

Recoveries for pirimiphos-methyl on small grains (wheat, sorghum grain, rice, and corn) fortified at levels of 0.1 to 10 ppm ranged from 80 to 130%. Recoveries for the des-ethyl metabolite on small grains fortified at levels of 0.05 to 1.0 ppm ranged from 60 to 132%. The petitioner reports that recoveries in samples requiring cleanup by acetonitrile-n-hexane partition such as milk and animal tissues are usually of the order 80 ± 10%; individual recovery values and sample chromatograms were not submitted. Recoveries of pirimiphos-methy and des-ethyl pirimiphos-methyl in egg yolks, egg whites, mixed flesh, and liver fortified at levels of 0.1 to 0.3 ppm ranged from 62 to 106%.

(II.) Method for the determination of hydroxypyrimidine metabolites of pirmiphos-methyl.

ICI's Provisional Residue Analytical Method-8182 is used for the determination of hydroxypyrimidine metabolites [2-diethylamino-6-methylpyrimidin-4-ol (Met. I), 2-ethylamino-6-methylpyrimidin-4-ol (Met. II), and 2-amino-6-methylpyrimidin-4-ol (Met. III)] on stored grains, and Plant Protection Division Residue Analytical Method No. 47 is used for the determination of the preceding metabolites in products of animal origin. The petitioner's summaries of the methodology used are as follows:

> "Grain samples are extracted by shaking with a mixture of 50% methanol:0.1 M HCl and hexane. The addition of hexane to the extraction solvent ensures removal of any pirimiphos-methyl (PP511) or phosphorothioate containing metabolites present on the grain. Following centrifugation an aliquot of the aqueous phase is evaporated to remove the methanol. The aqueous extracts are then neutralised and buffered to pH7 prior to ethyl-acetate and butanol partitions using Extrelut® columns. Compounds I and II are partitioned into 1% butanol: ethyl-acetate and compound III is partitioned into pure butanol. The partitioned extracts are subjected to adsorption chromatography to remove further interfering coextractives. The final determination is made by:
>
> A. High performance liquid chromatography using UV absorbance monitoring. This technique is suitable for compound I only.
>
> B. Gas-liquid chromatography using a nitrogen selective detector after the formation of trimethylsilyl derivatives. This procedure is suitable for all the hydroxypyrimidines."
>
> "Tissue samples are extracted by homogenisation in

the presence of 50% methanol:2M HCl. Following centrifugation an aliquot is shaken with hexane (to separate phosphorothioate containing pyrimidines) and then evaporated to remove the methanol. The aqueous extracts are refluxed to hydrolyse any hydroxypyrimidine conjugates. The hydrolysed extracts are then neutralised and buffered prior to butanol partition using Extrelut columns. The partitioned extracts are subjected to adsorption chromatography to remove further interfering coextractives. The final determination is by gas chromatography - mass spectrometry operated in the selected ion monitoring mode following the formation of trimethylsilyl derivatives. To compensate for small variations in the formation of the ditrimethylsilylated derivative of compound III in the presence of some substrates, the use of an analogous hydroxypyrimidine (R31680 2-amino-5,6-dimethylpyrimidin-4-ol) as an internal standard is recommended."

"Milk is extracted by blending samples (20g) with concentrated hydrochloric acid ($5cm^3$), methanol ($25cm^3$) and hexane ($20cm^3$). An aliquot of the aqueous phase is evaporated to remove methanol then neutralised and buffered for butanol partition. There is no hydrolysis step for milk as there is for tissues.

The hydrolysis step is also omitted from the procedure for egg analysis. Furthermore, the extraction procedure for eggs involves blending in a solvent mixture containing a higher proportion of methanol (90% methanol:10% 2M HCl) to encourage protein precipitation."

In an amendment (dated 3/10/83) to co-pending petition #9G2200, the petitioner injected an acid hydrolysis step prior to the ethyl acetate and butanol partitions in the above methodology used for grain samples.

Recoveries obtained from small grains (wheat, sorghum, rice, and corn) fortified at levels from 0.2 to 2.0 ppm ranged from 82 to 128% for Met. I, 57 to 105% for Met. II, and 55 to 91% for Met. III.

Recoveries (Mets. I, II, and III) from cow and chicken tissues fortified at levels from 0.1 to 5.0 ppm ranged from 47 to 100%.

Recoveries (Mets. I, II, and III) from cow milk fortified at levels from 0.0025 ppm to 1.0 ppm ranged from 55 to 119%, and recoveries from eggs fortified at a level of 0.05 ppm ranged from 65 to 118%.

Comments on the proposed methodology.

We can not conclude, at this time, that adequate methodology is available for regulatory purposes. Our conclusion depends on the outcome of the petitioner's further work on the identification of residues in plants and animals.

The petitioner should be informed, however, that if we did accept in this petition where pyrimidine metabolites are extracted from stored grains by a "surface/shaking" extraction technique (see Method No. 47), we would not accept this technique for extracting samples resulting from the field use of pirimiphos-methyl. Further, there will be a need for method trials once that the nature of the residue has been fully understood and a conclusion has been drawn on the suitability of the methodology for regulatory purposes. Also, the petitioner should delete the need for an internal standard from his methodology. Internal standards are considered undesirable for regulatory purposes.

Residue Data

The residue data involving the proposed use on farmers stock peanuts were submitted in PP#9G2154 and FAP#9H5201. For convenience, our (R.B. Perfetti and R.J. Hummel) review (dated 3/29/79) of the residue data is restated below:

"Following treatment with pirimiphos-methyl, 22 samples of farmers stock peanuts were ground up, analyzed and held in frozen storage (-20°C). Reanalysis after 3 years frozen storage showed a mean loss of only 10%.

Data reflecting residues of pirimiphos-methyl, per se, in or on whole peanuts were submitted from several studies. Farmers stock peanuts entering storage were sprayed with pirimiphos-methyl to give initial concentrations of 10-50 ppm (proposed, 20 ppm). In most studies, initial residues on whole peanuts were within 10% of the treatment rate. In one study, initial residues averaged 25.5 ppm and ranged from 13.1-38.6 ppm at a treatment rate of 20 ppm. The half life of parent compound on whole peanuts was ca. 6 months.

Data reflecting residues of pirimiphos-methyl, per se, in or on various fractions of peanuts were submitted from 4 studies. As expected, the bulk of the residue is on the hulls. In a study with segregation I peanuts (the best grade) initial residues on hulls were ca. 60 ppm following a 20 ppm nominal treatment. Residues in peanut meats were relatively constant at ca. 1 ppm over a 12 month storage interval. In studies with segregation II and III peanuts which had been treated at 20 ppm and stored ca. 6 months, residues on the hulls ranged from ca. 10-28 ppm and were up to ca. 5X those on whole

peanuts. Residues (parent compound) on cracked peanuts (meat) ranged from ca. 5-10 ppm. The relatively high levels on peanut meats is apparently due to the high proportion of unshelled and split peanuts in segregations II and III. These, of course, would have direct contact with the treatment spray.

We conclude that the above studies adequately demonstrate that residues in or on peanuts and peanut hulls resulting from the proposed use will not exceed the proposed tolerance. The lack of residue data for the metabolites of pirimiphos-methyl is of little concern as the proposed tolerance levels are adequate to cover initial residues.

In separate studies, the segregation II and segregation III peanuts above were processed in commercial oil processing and refining plants. Residues in the crude oil ranged from ca. 6-36 ppm and were ca. 3X those in cracked peanuts. Residues were reduced ca. 50% in the final refining procedure. Therefore, the residue level in the finished oil was ca. 2X that in the starting peanuts. Peanut meal contained no detectable residues (<0.1 ppm) and soapstock, only 0.3 ppm.

The above studies indicate that pirimiphos-methyl is relatively stable under peanut processing conditions and partitions essentially exclusively in the oil. We conclude that the proposed food additive tolerance of 50 ppm for residues in peanut oil is appropriate. Again, the lack of data for the metabolites is of little consequence as residues concentrate by the theoretical maximum in going from peanuts to peanut oil.

In a separate study it was shown that there was no concentration in residues in going from blanched peanuts to peanut butter."

We confirm our conclusions as stated above, i.e., the proposed pirimiphos-methyl tolerance levels (peanuts-25 ppm, peanut hulls-125 ppm, and peanut oil-50 ppm) are adequate to cover initial residues as applied to the peanuts; the reason being that these are calculated residue (tolerance) levels. Even though the tolerances are numerically adequate, the tolerance expression may need to be revised to include additional residues.

Meat, Milk, Poultry, and Eggs

Two feeding studies, one discussing the hydroxypyrimidine metabolites in the tissues and milk of cows and the other discussing the hydroxypyrimidine metabolites in the tissues and eggs of hens, were submitted in the present petition; they will be discussed near the end of this section.

Feeding studies on cows, pigs, and chicken were also discussed in our (R. Perfetti and R. Hummel) review (dated 3/29/79) of PP#9G2154.

The study on cows involved feeding 4 groups of 3 cows ca. 5, 15, and 50 ppm of pirimiphos-methyl in the diet for 30 days. Within 4 hours, 2 cows from each group were slaughtered and the remaining cows were held for 10 days without further medication and then slaughtered. Milk was sampled three times each week. After slaughter, liver, kidney, fat, heart, and muscle were sampled.

The analytical procedure used for milk and tissue measured parent, desethyl pirimiphos-methyl (II) and oxy pirimiphos-methyl (III). The stated method sensitivity was 0.005 ppm for all three compounds, but control values ranged up to 0.009 ppm. Residues in milk in excess of controls were found only at the two highest feeding levels. Maximum values in milk were 0.016 and 0.033 ppm for the 15 and 50 ppm feeding levels respectively. All analyses for II and III in milk, butter and tissue were <0.005 ppm. Residues in butter from the milk of the cow fed at the 50 ppm level ranged from 0.021 to 0.037 ppm. In most cases, a 2X concentration in going from milk to butter was observed. Residues in kidney, liver, fat, and heart were all <0.01 ppm for all feeding levels. The maximum residue found in muscle tissue was 0.017 ppm for a cow fed at the highest level.

The pig study reflected feeding groups of 4 pigs pirimiphos-methyl at 3, 9, and 34 ppm in the diet for up to 29 days. A control group of two pigs was also used. One pig from each group was slaughtered on day 21 and a second pig from each group was slaughtered on day 29. After an 8-day recovery period the remaining pigs were slaughtered. Samples of heart, kidney, liver, lungs, muscle and fat were taken. Samples were analyzed for parent, oxy pirimiphos-methyl (III), desethyl pirimiphos-methyl (II), hydroxy pyrimidine (IV) and desethyl hydroxy pyrimidine (V). Stated limits of detection for I-III were 0.005 ppm and for IV and V, 0.01 and 0.04 ppm, respectively. No detectable levels of oxy pirimiphos-methyl were found in any fat samples at any level. Maximum residues of parent in fat at the medium and highest feeding levels were 0.008 and 0.05 ppm, respectively, the former being observed at 21 days and the latter at 29 days. Maximum residues of the desethyl pirimiphos-methyl (II) in fat were 0.016 ppm at the 9 ppm feeding level and 0.036 ppm at the 34 ppm level. Again, the former value was observed at 21 days and the latter at 29 days. After an 8-day recovery period all residues in fat were <0.005 ppm for all feeding levels. The petitioner states that all levels of parent, II or III were <0.005 ppm in all other tissues. All levels of hydroxy pyrimidine (IV) and desethyl hydroxy pyrimidine (V) were given as <0.04 ppm and <0.01 ppm, respectively.

The 30-day cow feeding study submitted in the present petition involved pirimiphos-methyl feeding levels of 0, 10, 30 and 100 ppm. The petitioner indicates that the average intake, estimated using a trapezoidal rule calculation, was actually found to be 0, 8.3, 31, and 94 ppm.

Only the residues of the three hydroxypyrimidine metabolites [2-diethylamino-6-methylpyrimidin-4-ol (Met. I), 2-ethylamino-6-methylpyrimidin-4-ol (Met. II), and 2-amino-6-methylpyrimidin-4-ol (Met. III) found in tissues and milk resulting from the 94 ppm feeding level were reported. In milk, metabolites I and III did not exceed 0.01 ppm; metabolite II was found at a maximum level of 0.02 ppm. In muscle, none of the hydroxypyrimidine metabolites exceeded a level of 0.01 ppm. In liver, the maximum levels for metabolites I, II, and III were 0.03, 0.02, and 0.02, respectively. In kidney, the maximum levels for metabolites I, II, and III were 0.16, 0.14, and 0.05 ppm, respectively.

Our Comments/Conclusions on the Residues Found in Meat and Milk Commodities.

The animal feed items involved in this petition and a co-pending petition (PP#3F2897) are peanuts, peanut meal, hulls, and soapstock, corn grain, rice, grain sorghum, wheat grain, rice milling fractions, and wheat milling fractions; at this time, no other proposed permanent tolerances on feed items have been recommended for establishment by RCB. If we assume that horses, for example, were fed a diet comprising 50% corn, 5% rice, 20% wheat bran and 25% peanut hulls, then the dietary burden would be approximately 47 ppm pirimiphos-methyl residues. If dairy cows were fed a diet consisting of 10% cull peanuts, 25% rice bran, 25% wheat milled byproducts, and 40% corn grain, the dietary burden would be approximately 32 ppm pirimiphos-methyl residues.

At this time, we can not draw conclusions on the adequacy of the proposed meat, milk, fat, and meat byproducts tolerances; we need to know more about the nature of the residue in plants and animals. If metabolism is different in plants versus animals, a feeding and metabolism study with the plant residues of concern may be needed.

Also, the petitioner should note that there was a 2X concentrate of residues going from milk to butter. Therefore, there may be a need to propose a tolerance on a milk fat basis.

Feeding studies on poultry were also discussed in our (R. Perfetti and R. Hummel) review (dated 3/29/79) of PP#9G2154.

Three chicken feeding studies were reported. The first reflected feeding groups of 3 hens 1, 4 and 8 ppm of pirimiphos-

methyl in the diet for 18 days. No control hens were used. Maximum residues of parent in eggs were 0.008, 0.005, and 0.005 ppm for the 1, 4 and 8 ppm feeding levels, respectively. The petitioner states that no evidence for the presence of oxy pirimiphos-methyl (III) was obtained. The stated limit of detection of the method was 0.001 ppm. No analysis of tissues was made. Eggs fortified with parent compound at 0.01 and 0.1 ppm level gave recoveries of 80% of added material after 13 days storage at 4°C and at -17°C. Theoretical maximum levels in eggs would therefore be 0.01, 0.006 and 0.006 ppm for the 1, 4 and 8 ppm feeding levels, respectively, since these eggs were stored for up to 14 days before analysis.

The second chicken study involved feeding groups of 4 roosters and 35 hens pirimiphos-methyl at 0, 4, 12, and 40 ppm for 28 days. Eggs were sampled at 3, 7, 14, 21, 28 and 35 days. Five birds from each group were slaughtered on days 21, 28 and 35 and liver and mixed flesh were analyzed for parent, oxy pirimiphos-methyl (III), and desethyl pirimiphos-methyl (II). Residue of parent in egg yolk and whites ranged from <0.008 ppm at the lowest feeding level to 0.04 ppm at the highest feeding level. Residues of III in whites and yolks were all given as <0.008 and <0.02 ppm, respectively.

Residues of II in whites and yolks were all given as <0.006 and <0.016 ppm respectively. Residues of all three compounds in liver ranged from <0.004 ppm to less than 0.04 ppm. Maximum residues for parent in mixed tissue was 0.008 ppm. Residue values for III and II were given as <0.008 to <0.027 ppm.

Another chicken study involved feeding groups of 30 broiler chicks pirimiphos-methyl at 4, 8, 16, 32, and 48 ppm in the diet for 10 weeks. Birds were sampled within one hour of cessation of medication at week six and at the end of the trial, skin, fat and muscle were analyzed for parent, oxy pirimiphos-methyl (III) and desethyl pirimiphos-methyl (II). The petitioner states that no detectable residues of II or III were found in any of the samples.

Residues of parent in muscle tissues were given as non-detectable. Residues of parent in the skin were given as non-detectable to <0.01 ppm. Maximum residues of parent were found in fat and reached 0.017 ppm at the highest feeding level.

Poultry feeding study data submitted in the present petition show only the levels of the hydroxypyrimidine metabolites in the meat, liver, and eggs of poultry that were fed 0, 3.3, 11, and 38 ppm pyrimiphos-methyl in their diets for 28 consecutive days (preceding ppm levels estimated using a simple trapezoidal rule calculation; actually levels of

pyrimiphos-methyl incorporated into the feed were 0, 5, 15, and 40 ppm).

The residues (designated as metabolites I, II, and III in this report) found in the analyzed poultry commodities are given below:

		ppm range		
Commodity	Feeding level, ppm	Met. I	Met. II	Met. III
Breasts (composite)	Control	<0.01	<0.01	<0.01
	3.3	<0.01	<0.01	<0.01-0.08
	11	<0.01	<0.01	0.27-0.34
	38	<0.01	<0.01	0.47-0.96
Breast (individual)	38	<0.01	<0.01	0.6-1.3
Liver (composite)	Control	<0.01	<0.02	<0.01
	3.3	<0.01	<0.02	<0.01
	11	<0.01	<0.01	0.01-0.03
	38	<0.01-0.02	<0.01-0.06	0.01-0.05
Eggs (composite)	11	<0.01	<0.01	<0.01
	38	<0.01	<0.01-0.04	<0.01-0.03

Met. I = 2-diethylamino-6-methylpyrimidin-4-ol
Met.II = 2-ethylamino-6-methylpyrimidin-4-ol
Met.III= 2-amino-6-methylpyrimidin-4-ol

Our Comments/Conclusions on the Residues Found in Poultry and Egg Commodities.

The poultry feed items involved in this petition and a co-pending petition (PP#3F2897) are whole peanuts, peanut meal and soapstock; rice grain, bran, and polishing; sorghum grain; wheat grain and milled byproducts; and corn grain. If we assume that the dietary intake of poultry consisted of 10% peanut meal, 40% rice milling fraction, and 50% grain sorghum, then the dietary burden would be approximately 30 ppm.

In our review of PP#9G2154, we observed that hens fed 32 ppm of ^{14}C-pirimiphos-methyl for 7 days showed maximum radioactive residues in tissue of 0.41 ppm. Gas chromatographic analysis of these tissues showed no parent or oxy pirimiphos-methyl. The maximum residue (by radioactive measurement) occurring in eggs was 0.15 ppm. Gas chromatographic analysis of the eggs showed a maximum of 0.009 ppm of parent.

We can not draw any conclusions on the adequacy of the proposed tolerances on poultry meat and eggs until those questions relating to the nature of the residue in plants and animals/poultry have been resolved. Also, the petitioner should be informed that we will need residue data on poultry fat. Tolerances will need to be proposed on poultry meat by-products and poultry fat.

Other Comments

An International Residue Limit Status sheet is attached to this review. No Canadian and Mexican limits/tolerances have been established on peanut and animal commodities. We observed in WHO's 1974 Evaluation of Some Pesticide Residues in Foods that the (Codex) use on peanuts is similar to the U.S. use on peanuts. However, the Codex tolerances are much lower than the proposed U.S. tolerances, presumably, because the Codex tolerances do not include the hydroxypyrimidine metabolites.

TS-769:RCB:J.Onley:mch:CM#2:RM810:X77377:10/25/83

cc: R.F., Circu., J. Onley, Thompson, FDA, TOX, EEB, EAB
PP#3F2896/FAP#3H5398

RDI: R. Quick, 9/28/83; R. Schmitt, 9/29/83

INTERNATIONAL RESIDUE LIMIT STAT

CHEMICAL Pirimiphos-methyl

PETITION NO 3F2896/3H5398

CCPR NO. 86

Reviewer: J. Onley

F. A. 9/21/83

Codex Status

☐ No Codex Proposal Step 6 or above

Proposed U. S. Tolerances

Residue (if Step 9): sum of pirimiphos-methyl, its oxygen analogue and N-desethyl-pirimiphos-methyl, expressed as pirimiphos-methyl 1/

Residue: Pirimiphos-methyl and metabolites

Crop(s)	Limit (mg/kg)
peanuts (whole)	50
peanut (kernels)	5
milk	0.05 2/
meat, carcase	0.05 3/
peanut hulls	50
poultry	3/
eggs	0.05 2/
peanut oil	10

Crop(s)	Tol. (ppm)
Peanuts	25 ppm
Peanut hulls	125 ppm
Milk	0.5 ppm
Meat, fat and meat byproducts of cattle, goats, hogs, horses, and sheep (except liver and kidney)	0.15 ppm
Liver/liver byproducts	1.0 ppm
Kidney/kidney byproducts	2.0 ppm
Poultry	4.0 ppm
Eggs	0.5 ppm
Peanut oil	50 ppm

CANADIAN LIMIT

Residue: _____

Crop	Limit (ppm)
none	

MEXICAN TOLERANCIA

Residue: _____

Crop	Tolerancia (ppm)
none	

Notes:
1/ whether U.S. tolerances may be expressed as Codex needs to be addressed and if not, the basis therefore.
2/ at or about the limit of determination.
3/ presumed to be included under carcase meat.

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Chemical:	**Pirimiphos-methyl (ANSI)**
PC Code:	**108102**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**10/26/83**
File ID:	**00000000**
Accession Number:	**412-03-0015**

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